Effect of ZD4190, a VEGF receptor tyrosine kinase inhibitor, on endothelial permeability in human tumour xenografts

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BACKGROUND

The induction of new blood vessels from existing host vasculature (angiogenesis) is essential for supporting solid tumour growth [1]. Vascular endothelial growth factor (VEGF) is a key angiogenic factor which stimulates endothelial cell proliferation and migration. VEGF also contributes to tumour progression by increasing the permeability of the tumour vasculature, thereby facilitating extravasation of macromolecular proteins and aiding the establishment of a stroma. It is estimated that VEGF is 50,000 fold more potent at increasing microvessel permeability than histamine [2]. ZD4190 is an novel inhibitor of VEGF receptor tyrosine kinase (RTK), with potential utility as an anti-angiogenic treatment for solid tumour disease. This compound has significant antitumour activity during chronic oral administration in a range of human tumour xenograft models.

OBJECTIVE

To test the hypothesis that acute ZD4190 treatment reduces vascular endothelial permeability in human tumour xenografts.

METHODS

PC-3 human prostate carcinoma cells were implanted into athymic mice (10^6 per mouse) and allowed to grow for 27-49 days to a volume of between 0.4 and 1.2 ml. Mice were randomised (according to tumour size and number of lobes) to receive vehicle or ZD4190. Three studies were performed, in which mice were treated with (i) 0 or 100; (ii) 0, 50 or 100; and (iii) 0, 12½ or 25 mg.kg^{-1} respectively. Each mouse was orally dosed 0 and 22 hours after randomisation. MRI ('Inova', Varian, B_0=4.7T) was performed 24 hours after randomisation under terminal anaesthesia (using 'Fluothane', Zeneca). A voxelwise pre-contrast T_1 measurement was performed by saturation recovery. Gadopentetate dimeglumine ('Magnevist', Schering), 0.3 mmol.kg^{-1}, was given as a bolus i.v. A 53-minute dynamic multislice T_1-weighted spin-echo sequence was then employed with TR=120ms; TE=10ms. There were five sagittal slices through the tumour to map the contrast agent uptake and washout; one transverse slice through the abdominal aorta and vena cava to measure the vascular input function (VIF); and one sagittal saturation slice applied through the heart and great vessels to suppress any contribution from fast in-flowing blood within the transverse slice. Time resolution was 16s, and voxel dimensions were 0.03 × 0.06 × 2 mm. Image analysis code was written using 'IDL', (Research Systems Inc). Voxelwise gadopentetate concentration was obtained from voxelwise ΔT_1^{-1}. VIF was fitted to a double exponential, and voxelwise K, the endothelial permeability surface product, was obtained according to the Tofts-Kermode model [3]. Data acquisition, analysis, and exclusion were performed blinded. The principal exclusion criterion was inadequate or absent VIF.

SIGNIFICANCE TESTING

Three analyses, each of which may be vulnerable to different confounds, were performed:

a) For each mouse the mean of the voxelwise K values was determined to give a tumour mean K, then Student's t-test was used to compare groups of ZD4190 and vehicle-treated mice;

b) For each mouse a cumulative, volume-weighted histogram of the voxelwise K values was obtained, then Student's t-test was used to compare K at 100 threshold values in ZD4190 and vehicle-treated mice;

c) As (b), but a permutation test [4] was used instead of 100 t-tests

RESULTS

After imposition of the exclusion criteria, data sets from 71 mice remained available for analysis. The mean inter-subject coefficient of variation was found to be 40%. Figure 1 shows tumour mean K as a function of dose. In mice treated with ZD4190 at 100 mg.kg^{-1}, K was reduced by 31%-47%; at 50 mg.kg^{-1}, K was reduced by 26%, while at lower doses there was no significant effect. All three methods of significance testing gave similar results.

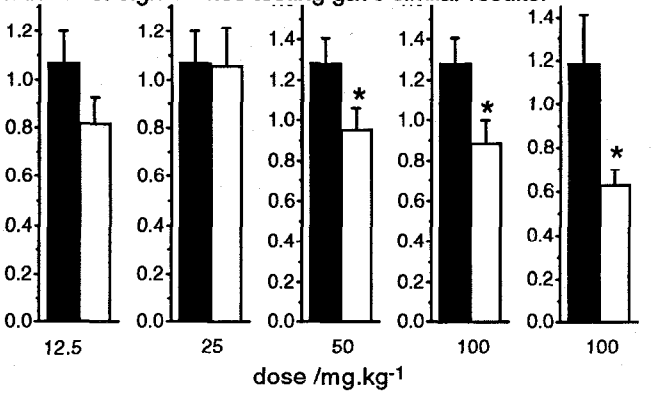

Figure 1. Tumour mean endothelial permeability surface product K /10^{-3}s^{-1} ± S.E.M. □, ZD4190 p.o.; ■, corresponding vehicle data; *, $P<0.05$

The data were further explored using ANOVA. There was, as expected, a significant correlation between K and dose, but no significant correlation with K was found for tumour volume, age or number of lobes.

DISCUSSION

There was a robust finding of reduced endothelial permeability surface product K by acute treatment with 50 and 100 mg.kg^{-1} of ZD4190. These doses also significantly inhibit tumour xenograft growth during chronic administration. The findings are consistent with the inhibition of VEGF RTK.

REFERENCES

1. Boehm, T., *et al. Nature*, 390:404 (1997)
2. Dvorak, H.F., *et al. Int. Arch. Allergy Appl. Immunol.*, 107: 233 (1995)
3. Tofts, P., Kermode, A.G. *Magn. Res. Med.* 17:357 (1991)
4. Siegel,S., Castellan Jr N.J. Nonparametric Statistics for Behavioral Sciences 2nd Ed. McGraw-Hill (1988)